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Concentration-response relationships for salicylate-induced ototoxicity in normal volunteers

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1 Ototoxicity is a common and troublesome side-effect of high-dose aspirin treatment but there has been little previous study of the relationships between the degree of ototoxicity and the plasma concentrations of salicylate.

2 In order to investigate the relationships between aspirin dose, total and unbound plasma salicylate concentrations and ototoxicity, eight normal volunteers were dosed with aspirin 1.95, 3.25, 4.55 and 5.85 g day^{-1} for 1 week at each dose level, the doses being administered in random order and double-blind, 2 weeks apart.

3 Ototoxic effects measured were hearing loss in decibels (dB) over six frequencies and tinnitus intensity, estimated both by electronic matching and a fixed interval scale (FIS). Measurements were taken after steady-state concentrations of salicylate had been achieved.

4 Total and unbound plasma salicylate concentrations increased disproportionately with increasing daily doses of aspirin. The increase in the unbound salicylate was relatively greater since the percentage of salicylate unbound in plasma increased over the dose range investigated from a mean of 3.9% to 10.4%.

5 Hearing loss and tinnitus intensity increased progressively with the aspirin dosage and increasing concentrations of total and unbound plasma salicylate concentrations. These ototoxic symptoms were observed at lower concentrations of total salicylate than previously reported.

6 There was a linear relationship between hearing loss and unbound salicylate concentations.

7 Further work is required to test the hypothesis that unbound plasma salicylate concentration is a better predictor of salicylate-induced ototoxicity than total plasma salicylate concentration.

Keywords salicylate ototoxicity deafness tinnitus unbound drug

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Introduction

Deafness and tinnitus are well recognized adverse effects of high-dose salicylate therapy. These ototoxic effects of salicylate are related to the plasma concentrations of salicylate (Myers *et al.*, 1965; Mongan *et al.*, 1973). Myers *et al.* (1965), examined hearing loss in 21 patients after the dosage of aspirin had been raised sufficiently for the patients to be aware of tinnitus and subjective hearing loss. Objective evidence of deafness was then found, with the plasma salicylate concentrations ranging from 230 to 650 mg l^{-1}. Mongan *et al.* (1965), similarly increased the dosage of aspirin until tinnitus was noted. Tinnitus occurred in all patients with normal hearing if the plasma concentrations of salicylate were raised sufficiently. Salicylate concentrations at which tinnitus was first reported ranged from 196 to 458 mg l^{-1} (mean 295 mg l^{-1}) but many patients with pre-existing hearing loss did not experience tinnitus despite very high plasma concentrations of salicylate. These results have been interpreted as indicating that there is a threshold plasma concentration below which ototoxicity does not occur. This threshold may vary in different patients but the minimum threshold for tinnitus was 196 mg l^{-1} (Mongan *et al.*, 1965) and for deafness was approximately 220 mg l^{-1} (Myers *et al.*, 1973). More recently, Halla & Hardin (1988) reported that tinnitus and deafness could occur below these threshold levels.

While ototoxicity is a definite side-effect of salicylate, formal dose-concentration-response relationships for salicylate-induced ototoxicity have not been performed previously. Preliminary observations on some of our patients taking salicylates for rheumatic diseases and who were complaining of ototoxicity, suggested to us that unbound salicylate concentrations may be a better correlate with ototoxicity than total salicylate concentrations. As a first step in a more thorough investigation of the ototoxicity of salicylate, we conducted a double-blind study of the relationships between aspirin dosage, plasma salicylate concentrations and the degree of ototoxicity in healthy subjects.

Methods

Subjects

Eight healthy male volunteers, aged 22 to 46 years were admitted to the trial after giving informed consent. The study was approved by the Ethics and Research Committee of St Vincent's Hospital. Each subject had an audiological examination for hearing loss and was assessed as normal. The subjects neither smoked nor drank alcohol during the periods they were taking aspirin and no subject was taking any other medication.

Dosage schedules

Each subject received four dose levels of aspirin for 7 days (1.95, 3.25, 4.55 and 5.85 g day^{-1}). Aspirin was administered as slow-release tablets (SRA, Boots Co., Sydney; 650 mg $tablet^{-1}$). The order of dosage was determined by a Latin square design balanced for order effects (Williams, 1949) and the trial was conducted double-blind. To achieve this, identical placebos were prepared. At each dose level, three tablets were taken strictly every 8 h, the first dose of the day being taken between 06.00 and 07.00 h. Compliance was promoted by the use of Dosette compliance boxes. At least 2 weeks (range 13–21 days; mean ± s.d., 14.6 ± 1.9 days) separated the periods of aspirin treatment, and audiological testing was performed to ensure normal hearing prior to commencing a new dose level. In fact, no subject had abnormal hearing detected between the aspirin treatment periods. Audiological laboratory examination was performed on the seventh day of each treatment period between 10.00 h and 11.00 h. Hearing thresholds and tinnitus loudness and pitch were assessed. Two venous blood samples (10 ml) were taken no more than 1 h apart on day 7 and this sampling interval encompassed the time at which the formal audiological testing was conducted. Blood samples were collected into Vacutainer glass tubes containing 15 mg of sodium edetate.

Audiological assessments

Hearing thresholds were measured for both ears in decibels, according to the hearing level convention (dB HL), using pure tones generated by an audiometer (Madsen audiometer, 0B822, Denmark) at frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hz.

A tinnitus synthesizer (SG-1; Norwestern Acoustics) was used to allow subjects to match the pitch in hertz (Hz) and loudness in dB SPL (sound pressure level convention) of any tinnitus experienced. The subjects adjusted the loudness and the pitch of the pure tone produced by the tinnitus synthesizer to match, as closely as possible, the tinnitus they were currently perceiving. Many of the subjects considered that tinnitus was apparently composed of several tones and the dominant tone experienced was used to estimate pitch and loudness of the tinnitus.

Subjects also estimated the pitch and loudness of their tinnitus using fixed-interval scales. Loudness was scored from 1–5 inclusive on an integer scale and similarly, pitch was scored 1–4. Subjects made these assessments three times a day during the 7 day dosing schedules, on awakening, mid-afternoon and before retiring at night. All subjects were given basic (pre-trial) and refresher (between dosage levels) training in estimating pitch and loudness of pure tones which were presented to the subjects in random order. The five levels of loudness used in the training were 0, 10, 20, 30 and 40 dB HL and the four levels of pitch were 750, 1500, 2250 and 3000 Hz. Initially, exercises were performed varying either pitch or intensity of the pure tone, the other parameter remaining fixed. Subsequently, random mixtures of pitch and intensity were presented to the subjects for simultaneous indentification. Subjects were not entered into the study until they achieved good accuracy (> 70%), for identifying the correct level of pitch and loudness in a 40 min training session. This degree of accuracy was usually achieved after three sessions conducted on 3 separate days. Two refresher training sessions were held on 2 separate days prior to each new dosage level.

Plasma salicylate measurements

Plasma salicylate concentrations were measured fluorimetrically (Hitachi 204 fluorescence spectrophotometer) according to the method of Rowland & Riegelman (1967).

Plasma samples were dialysed against isotonic, phosphate-buffered (pH 7.4) saline in order to measure unbound plasma salicylate concentrations. Approximately equal volumes of plasma and buffer were placed in 1 ml perspex dialysis cells separated by Visking 22/32 dialysis membrane. The dialysis cells were incubated at 37° C for 18 h. The total salicylate concentrations in the plasma and buffer compartments were measured by the method of Rowland & Riegelman (1967). A correction for the net fluid shift into the plasma compartment during dialysis was made by the method of Tozer *et al.* (1983), the degree of fluid-shift being determined by the concentration of Evans Blue in the plasma compartment after dialysis.

Data analysis and statistics

Plasma total and unbound salicylate concentrations reported for each individual at each dosage level are the mean of two results from plasma samples taken about 1 h apart and encompassing the audiological assessments on day 7 of each dosing period. The difference between these sample pairs was 4.9 ± 3.7% (mean ± s.d.) and was never greater than 14%.

Hearing loss is expressed as the mean decrease from pretreatment values of hearing threshold across the six frequencies tested in both ears.

Tinnitus intensity scores, measured by the fixed interval scales, are the grand mean of the scores of the three estimations made by the subjects on days 4, 5 and 6 of each dosing period. Tinnitus intensity showed no significant increase after day 4.

Mean ± s.e. mean for total salicylate, free salicylate, hearing loss and tinnitus for each of the four salicylate doses are presented. Analysis of variance was used to compare the means for each of these variables across doses and to examine for any treatment order effects or interactions.

Results

Total and unbound plasma salicylate concentrations increased disproportionately with increasing daily-doses of aspirin (Table 1).

The increase in the unbound salicylate was relatively greater since the fraction of salicylate unbound in plasma increased over the dose range investigated from a mean of 3.9% to 10.4%.

The hearing loss and tinnitus intensity, measured by both the matching and the fixed interval scale techniques, for each of the four dosing levels of aspirin are presented in Table 1. Basal hearing intensity assessments revealed an average score ± s.d. per ear of 40 ± 24 dB HL across the six frequencies tested (range 12.5–90). The results on tinnitus pitch are not presented in detail. The pitches recorded by the subjects using the matching technique were in the range 900 to 14,500 Hz and there was no correlation with dosage of aspirin or plasma concentrations of salicylate. The pitches used to train subjects in the use of the fixed interval scale were generally below those experienced.

Hearing loss and tinnitus were dependent on the dose of aspirin and generally increased with each increment of dose (Tables 1 and 2) but there was no significant increase between baseline and 1.95 g day^{-1}. There was a linear relationship between hearing loss and unbound salicylate concentrations in plasma (Figure 1a). The intercept on this plot was not significantly different from zero indicating that there was a proportionality between hearing loss and the unbound concentrations in plasma. Hearing loss increased with increasing total plasma concentrations of the drug but a linear relationship was less apparent

Table 1 Mean (s.e. mean) of salicylate concentrations and parameters of ototoxicity observed with daily aspirin dosages of 1.95, 3.25, 4.55 and 5.85 g day^{-1}

Aspirin (g day^{-1})	*Total SAL (mg l^{-1})*	*Unbound SAL (mg l^{-1})*	*Hearing * loss (dB HL)*	*Tinnitus # (FIS)*	*Tinnitus § (dB SPL)*
1.95	43.54 (4.7)	1.69 (0.3)	4.38 (3.9)	0.86 (0.3)	25.50 (6.0)
3.25	110.64 (15.1)	6.64 (1.6)	11.56 (3.3)	1.81 (0.4)	36.38 (6.5)
4.55	216.11 (15.1)	22.53 (2.9)	46.56 (9.0)	2.59 (0.3)	51.00 (3.8)
5.85	324.08 (20.5)	46.18 (6.6)	112.50 (17.6)	3.21 (0.2)	62.13 (5.2)

*Change from baseline; mean hearing loss in dB per frequency examined (six frequencies for each ear were tested). # Tinnitus estimated using a fixed interval scale and performed at home (FIS). § Tinnitus in dB SPL estimated by matching with pure tones.

Table 2 The effect of aspirin upon parameters of ototoxicity; contrasts between doses. Significance levels were derived from analysis of variance appropriate for the latin square employed and contrasts between dose levels were examined by Newman Keuls least significant difference test (Snedecor & Cochran, 1967).

		(P values)	
Aspirin doses (g day^{-1})	*Hearing loss (dB HL)*	*Tinnitus (FIS)*	*Tinnitus (dB SPL)*
Across all doses	0.0001	0.001	0.0005
1.95 *vs* 3.25	NS ($P > 0.20$)	< 0.05	NS ($0.2 > P > 0.1$)
1.95 *vs* 4.55	< 0.05	< 0.05	< 0.05
1.95 *vs* 5.85	< 0.05	< 0.05	< 0.05
3.25 *vs* 4.55	< 0.05	< 0.05	NS ($0.1 > P > 0.05$)
3.25 *vs* 5.85	< 0.05	< 0.05	< 0.05
4.55 *vs* 5.85	< 0.05	NS ($0.1 > P > 0.05$)	NS ($0.2 > P > 0.1$)

NS = not significant.

from inspection of the data (Figure 1b). The pattern of hearing loss sustained as a result of salicylate therapy was, in general, a global decrement across all frequencies examined.

Tinnitus intensity generally increased throughout the range of total and unbound salicylate concentrations in plasma (Figures 2a and b) although the patterns for the different subjects were quite variable. Tinnitus intensity measured by the fixed interval scale and matching methods showed similar patterns with respect to plasma concentrations of salicylate but only the data on tinnitus measured by the fixed interval scale method is shown (Figures 2a and b). There was an approximately linear relationship apparent between tinnitus intensity and the total plasma concentrations of salicylate (Figure 2a) but there appeared to be a trend towards a less than proportional increase in tinnitus intensity with increasing unbound plasma concentrations (Figure 2b). However, the variations in the data prevented definite conclusions about the mathematical relationships between tinnitus intensity and the plasma concentrations of salicylate.

Discussion

This study presents the relationship between ototoxicity and salicylate dose and total and unbound plasma salicylate concentrations in normal human volunteers across a wide range of salicylate concentrations. Salicylate-induced hearing loss and tinnitus was demonstrated at

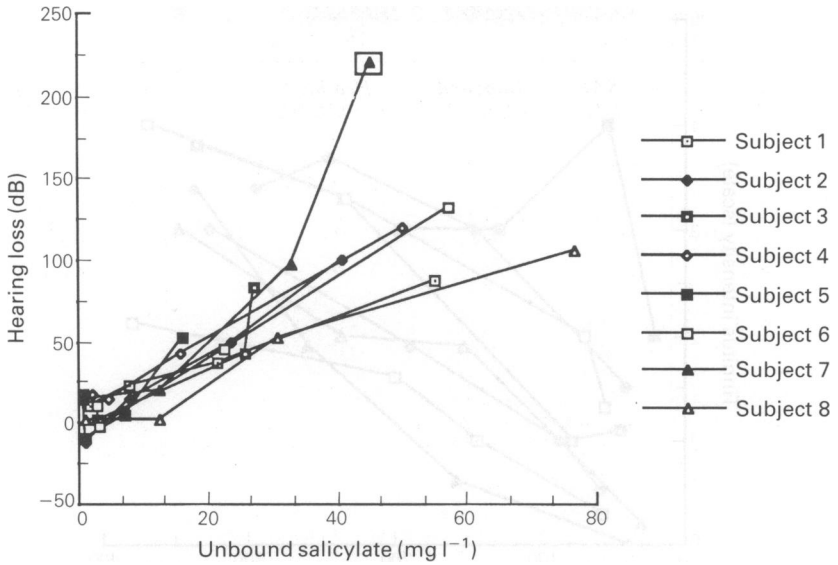

Figure 1 a) Relationship between hearing loss (HL) and unbound concentrations of salicylate in plasma (C_u). Least squares, linear regression of these data, excluding the outlier (boxed), gave the relationship: HL = 1.9 C_u + 3.7 with a correlation coefficient of 0.92 ($P = 0.0001$) and the 95% confidence intervals for the intercept 3.7 mg l^{-1} are −4.65 to 12.03 which overlap zero.

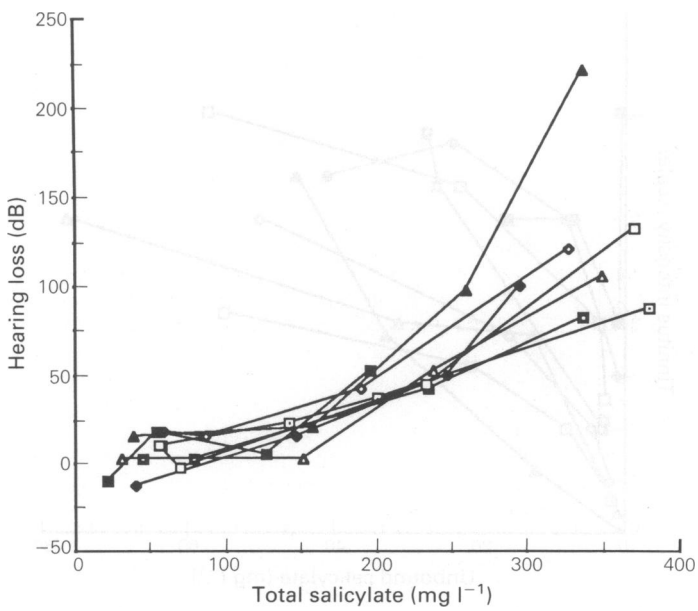

Figure 1 b) Relationship between hearing loss and total plasma salicylate concentrations for all subjects for all doses.

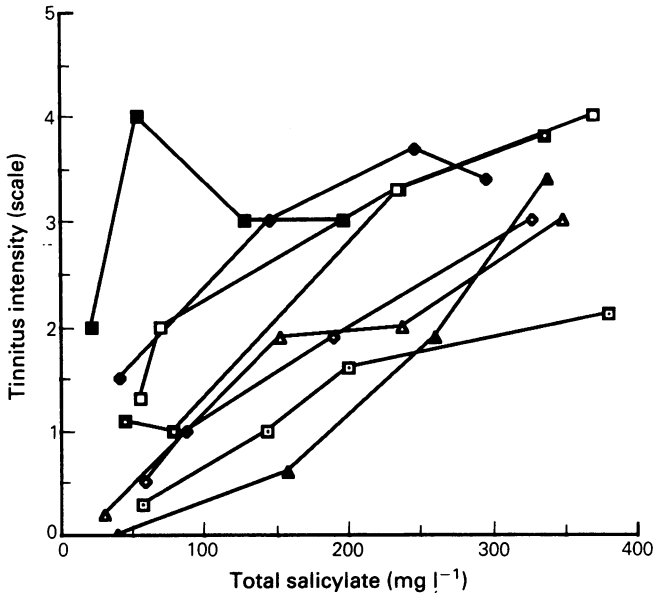

Figure 2 a) Relationship between tinnitus intensity (fixed interval scale) and total concentrations of salicylate in plasma.

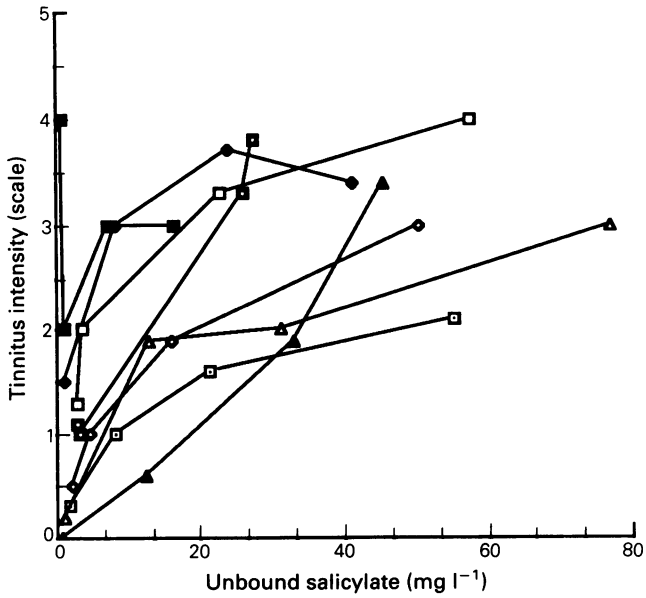

Figure 2 b) Relationship between tinnitus intensity (fixed interval scale) and unbound concentrations of salicylate in plasma. Symbols as for Figure 1.

lower doses and total plasma concentrations of salicylate than have previously been reported. A graded increase in the intensities of both hearing loss and tinnitus with increasing doses and plasma concentrations of salicylate was demonstrated for the first time as were the relationships between unbound plasma salicylate concentrations and hearing loss and tinnitus.

In one of the few previous studies on the hearing loss induced by salicylates, Myers *et al.* (1965) examined the changes in audiograms produced by high doses of salicylates. These investigators only measured hearing loss when it had developed to an extent which could be detected by the patients. As a result, the hearing deficit recorded by Myers *et al.* (1965) was, on average, greater than that achieved in our study and the plasma concentrations of salicylate were correspondingly higher. In fact, the relationship between hearing loss and salicylate concentrations reported by Myers *et al.* (1965) forms a continuous regression with the data obtained in the present study (Figure 3) and it is evident that some hearing loss can be produced well below the minimum salicylate concentration (220 mg l^{-1}) found in the study of Myers *et al.* (1965).

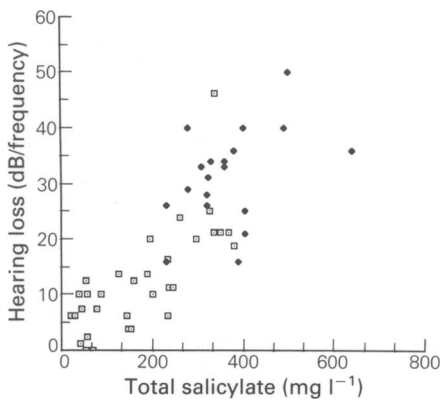

Figure 3 Relationship between hearing loss at 1000 and 2000 Hz and total plasma concentration of salicylate showing that the data of Myers *et al.* (1965) (◆) are continuous with the data from the present study (□).

The level of hearing deficit sustained with salicylate concentrations below 200 mg l^{-1} is small but we have demonstrated that there is no threshold for this effect. Thus, at an average total salicylate concentration of 110 mg l^{-1} the hearing loss at any given frequency is of the order of 12 dB (Table 1). This order of hearing deficit is unlikely to be clinically relevant in subjects with previously normal hearing but may be pertinent in those with previous hearing impairment. Halla & Hardin (1988) recently found that some hearing loss can be associated with plasma concentrations of salicylate below 200 mg l^{-1} although they did not report the extent of the deficit. Our results on hearing loss thus confirm and extend the data of Halla & Hardin (1988).

Our data on the intensity of tinnitus is less consistent than on hearing loss but the results indicate that there is a continuous increase in the intensity of tinnitus over the range of plasma concentrations studied (approximately 40–320 mg l^{-1}). Tinnitus intensity as a function of salicylate concentration has not been reported previously and it is apparent from this study that this symptom is a continuous variable. Our results conflict with the findings of Mongan *et al.* (1973) who reported that there was a threshold plasma concentration below which tinnitus did not develop. In their study, this threshold varied in different patients but was at least 196 mg l^{-1} and was higher in patients with pre-existing hearing loss. Again, our results confirm the findings of Halla & Hardin (1988) who reported that tinnitus could be associated with plasma concentrations of salicylate below 196 mg l^{-1}.

Peak plasma concentrations of salicylate achieved by single analgesic and antipyretic doses of aspirin (300–1000 mg) are in the range 30–100 mg l^{-1} while anti-inflammatory dosage is often adjusted to achieve plasma concentrations between 150 and 300 mg l^{-1}. The present study shows that hearing loss and tinnitus increase over the range of plasma concentrations of salicylate which are achieved by these therapeutic doses of aspirin. The plasma concentrations of salicylate attained by anti-platelet doses of aspirin are frequently below 10 mg l^{-1}. The degree of ototoxicity caused by these doses was not measured in our studies but would appear to be very low.

Our results do not indicate whether unbound plasma concentrations of salicylate are a better correlate of ototoxicity than the total plasma concentrations although it is anticipated that they should be. Further studies on subjects with a wide range of salicylate binding to plasma proteins is required to resolve this question.

The data of this study support the idea that appearance of ototoxicity indicates elevation of plasma salicylate concentrations, at least in trained normal volunteers. Additionally, the intensity of these ototoxic symptoms increases with increasing salicylate concentrations. The present work has shown that there is considerable intersubject variation in the response of the

human ear to particular concentrations of salicylate. This may be more noticeable in patient populations. Thus, Mongan *et al.* (1973), reported that those with preexisting hearing loss may not even register tinnitus despite very high plasma salicylate concentrations. Further work on the relationships between plasma salicylate concentrations and ototoxicity in patient populations is required and in particular, to test the hypothesis that unbound plasma salicylate concentration is a better predictor of salicylate-induced ototoxicity than total plasma salicylate concentration.

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References

Champion, G. D., Day, R. O., Graham, G. G. & Paull, P. D. (1975). Salicylates in rheumatoid arthritis. *Clin. Rheum. Dis.*, **1**, 245–265.

Halla, J. T. & Hardin, J. G. (1988). Salicylate ototoxicity in patients with rheumatoid athritis: a controlled study. *Ann. Rheum. Dis.*, **47**, 134–7.

Myers, E. N., Bernstein, J. M. & Fostiropolous, G. (1965). Salicylate ototoxicity. A clinical study. *New Engl. J. Med.*, **273**, 587–90.

Mongan, E., Kelly, P., Nies, K., Porter, W. W. & Paulus, H. E. (1973). Tinnitus as an indication of therapeutic serum salicylate levels. *J. Am. med. Ass.*, **226**, 142–145.

Rowland, M. & Riegelman, S. (1967). Determination of acetylsalicylic acid and salicylic acid in plasma. *J. pharm. Sci.*, **56**, 717–720.

Snedecor, G. W. & Cochran W. G. (1967). One way classifications. Analysis of variance. In *Statistical methods*, sixth edition, pp 258–298. Ames, Iowa, USA: The Iowa State University Press.

Tozer, T. N., Gambertoglio, J. G., Furst, D. E., Avery, D. S & Holford, N. H. G. (1983). Volume shifts and protein binding estimates using equilibrium dialysis: Application to prednisolone binding in humans. *J. pharm Sci.*, **72**, 1442–1446.

Williams, E. G. (1949). Experimental designs balanced for the estimation of residual effects of treatment. *J. sci. Res., Series A*, **2**, 149–168.

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